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The Effect of Methylmercuric Chloride, Cadmium Chloride, and Lead Nitrate on Six Biochemical Factors of the Brook Trout (Salvelinus fontinalis)

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16. ABSTRACT

Brook trout were exposed to experimental water concentrations of methylmercuric chloride at concentrations from 0.01 to 2.93 µg of Hg/liter, to cadmium chloride from 0.06 to 6.35 µg of Cd/liter, and to lead nitrate from 0.90 to 474 µg of Pb/liter. Exposures were for 2- and 8-week periods. Fish weight and length, hemoglobin, and blood plasma sodium, chloride, glucose, glutamic oxalacetic transaminase, and lactic dehydrogenase were measured. The following observed changes were statistically significant: increases in plasma sodium and chloride and decreases in hemoglobin and glutamic oxalacetic transaminase activity caused by lead; increases in plasma chloride and lactic dehydrogenase activity and a decrease in plasma glucose caused by cadmium; and increases in hemoglobin and plasma sodium and chloride caused by methylmercury. Comparisons were made between these biochemical findings and published data from bioassay and tissue-residue studies regarding the determination of threshold indices of toxicity.

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The Effect of Methylmercuric Chloride, Cadmium Chloride, and Lead Nitrate on Six Biochemical Factors of the Brook Trout (*Salvelinus fontinalis*)

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The Effect of Methylmercuric Chloride, Cadmium Chloride, and Lead Nitrate on Six Biochemical Factors of the Brook Trout (*Salvelinus fontinalis*). CHRISTENSEN, G., HUNT, E., AND FIANDT, J. (1977). *Toxicol. Appl. Pharmacol.* **42**, 523–530. Brook trout were exposed to experimental water concentrations of methylmercuric chloride at concentrations from 0.01 to 2.93 μg of Hg/liter, to cadmium chloride from 0.06 to 6.35 μg of Cd/liter, and to lead nitrate from 0.90 to 474 μg of Pb/liter. Exposures were for 2- and 8-week periods. Fish weight and length, hemoglobin, and blood plasma sodium, chloride, glucose, glutamic oxalacetic transaminase, and lactic dehydrogenase were measured. The following observed changes were statistically significant: increases in plasma sodium and chloride and decreases in hemoglobin and glutamic oxalacetic transaminase activity caused by lead; increases in plasma chloride and lactic dehydrogenase activity and a decrease in plasma glucose caused by cadmium; and increases in hemoglobin and plasma sodium and chloride caused by methylmercury. Comparisons were made between these biochemical findings and published data from bioassay and tissue-residue studies regarding the determination of threshold indices of toxicity.

The effects of pollutant chemicals upon aquatic and terrestrial populations should be evaluated in terms of toxicity, stimulation, inhibition, destruction, and alteration (Nelson, 1975). Both interdisciplinary and specialized research is needed for water pollution studies and should include both short- and long-term exposure experimentation. Because of many obvious serious limitations in carrying out long-term chronic toxicity studies for all of the great mass of chemical water pollutants for all species of aquatic animals, albeit that this may provide the most uncontroversial final results on toxic effects, it is of critical importance to establish some realistic, provisional, short-term screening toxicity tests. This is being done for the study of environmental agents suspected of being carcinogenic (Muul *et al.*, 1976; Bridges, 1976; Ames, 1977). This is also being tested with fish as affected by water pollutants by McKim (1977), who found for many water toxicants that a short exposure during the embryo–fry life stage of the fish provides bioassay data equivalent to those derived from parallel chronic exposure to adults, thereby providing toxic threshold values for fish after only a few weeks of study.

The early detection of specific molecular abnormalities in the tissue of aquatic as well as of terrestrial animals may provide an indication of exposure well before any gross signs become apparent (Palmes, 1976; Thomas *et al.*, 1973; Koeman and Strik, 1975).

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Such biochemical indices may prove to be of special value in signaling the development of sublethal abnormalities which could cause an animal population to be less efficient or effective in coping with the normal stress and strain of survival.

Being that water quality criteria, based upon extensive toxicity research, have now been established for many water pollutants for domestic drinking water and for some groups of aquatic animals, baseline ("no effect" or "safe") reference data are available against which toxicity indices from various methods and procedures can be correlated (USEPA, 1976). A reasonable correlation has been described between lethality due to chronic exposure (i.e., bioassay endpoint) and biochemical factors in an experiment with the brown bullhead (*Ictalurus nebulosus*) exposed to cupric sulfate for nearly 2 years (Christensen *et al.*, 1972). More recently, it was suggested that brook trout (*Salvelinus fontinalis*) alevins (fry) were more susceptible than embryos or adults to toxicity by methylmercuric chloride, cadmium chloride, and lead nitrate, as indicated by the analysis of several biochemical factors (Christensen, 1975). This biochemical experimentation on the early life stages was a component of three parallel long-term toxicity experiments recently concluded dealing with methylmercuric chloride (McKim *et al.*, 1976), cadmium chloride (Benoit *et al.*, 1976), and lead nitrate (Holcombe *et al.*, 1976). These three chronic toxicity experiments extended through three generations, covering all life stages and involved the determination of mortality, reproduction effects, and the absorption, distribution, and elimination rates of the toxicant.

As a part of these studies, certain biochemical and hematological parameters were studied. Yearling fish after 2 and 8 weeks of exposure in the above described studies were the subjects of the current report. The blood factors measured were hemoglobin and plasma sodium, chloride, glucose, GOT, and LDH.[1] Lengths and weights of the fish were also measured.

METHODS

Three previous reports (Benoit *et al.*, 1976; Holcombe *et al.*, 1976; McKim *et al.*, 1976) described the maintenance and exposure of the test fish and larvae, the chemical characteristics of the experimental water, the preparation and monitoring of the toxicants, and the physical characteristics of the experimental systems. In this study the measured toxicant concentrations (approximate) in the test water, expressed in micrograms of metal per liter, were: for MMC, 0.01 (control), 0.03, 0.09, 0.29, 0.93, and 2.93; for Cd, 0.06 (control), 0.50, 0.85, 1.65, 3.40, and 6.35; and for Pb, 0.90 (control), 34, 58, 119, 233, and 474.

Biochemical studies were carried out on yearling (first generation) trout after 2- and 8-week exposures to MMC (from 0.03 to 2.93 μg of Hg/liter, 0.00015 to 0.0146 mol/liter), Cd (from 0.06 to 6.35 μg of Cd/liter; 0.00053 to 0.0565 mol/liter), and Pb (from 0.90 to 474 μg of Pb/liter; 0.0043 to 2.29 mol/liter). The fish were anesthetized,[2,3]

[1] Abbreviations used: LC50 = lethal concentration causing 50% mortality; GOT = glutamic-oxaloacetic transaminase, L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1; LDH = lactic acid dehydrogenase, L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27; MMC = methylmercuric chloride; Cd = $CdCl_2$; Pb = $Pb(NO_3)_2$; ALAD = δ-aminolevulinic acid dehydratase, 5-aminolevulinate hydrolyase, EC 4.2.1.24.

[2] Ethyl-*m*-aminobenzoate methanesulfonic acid (MS-222), 100 mg/liter; Aldrich Chemical Co., Milwaukee, Wis.

[3] The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

lengths and weights were determined, and blood samples were taken from the caudal vasculature by heparinized needle and syringe. The procedures have been described in detail by McKim *et al.* (1970).

Hemoglobin analysis was carried out by the cyanmethemoglobin procedure (Frankel *et al.*, 1970) with 20 μl of blood and premeasured amounts of reagents.[4] Results were compared to a hemoglobin reference standard.[5] The blood samples were then centrifuged (5°C) to obtain the plasma, which was immediately analyzed or stored in plastic vials (−20°C) until analyzed (no longer than 1 week later). Plasma sodium concentrations were determined by atomic absorption spectrophotometry (Perkin–Elmer Corp., 1971), and plasma chloride was analyzed titrimetrically (Frankel *et al.*, 1970). In both tests, reagent-grade NaCl was used as a standard. Plasma glucose and LDH activity were determined by autoanalysis (Technicon Corp., 1971), as was plasma GOT activity (Babson *et al.*, 1962); control sera were used as standards.[6]

A statistical analysis of the data was carried out by testing control values against each treatment value according to a one-way analysis of variance with the F test, and significance was tested at $p < 0.05$ and < 0.01 (Steel and Torrie, 1960).

RESULTS

Determinations of brook trout weight, length, hemoglobin, and blood plasma sodium after 2 and 8 weeks of exposure to MMC, Cd, and Pb are summarized in Table 1. The hemoglobin concentration increased in response to MMC (2 weeks) and decreased with exposure to Pb (2 and 8 weeks). The plasma sodium ion concentration increased with exposure to MMC (2 weeks) and Pb (2 and 8 weeks). Weight and length of the fish were not affected.

The results of the analysis of blood plasma chloride, glucose, GOT, and LDH are shown in Table 2. Statistically significant increases were observed in the chloride concentration with exposure to MMC (2 weeks), Pb (2 weeks), and Cd (8 weeks). Glucose concentrations decreased with exposure to Cd (8 weeks), GOT activity decreased with exposure to Pb (2 and 8 weeks), and LDH activity increased with exposure to Cd (8 weeks).

These results are summarized and then correlated with related toxicity data as shown in Table 3.

DISCUSSION

In this experiment, a significant biochemical or blood parameter change was taken to indicate an early sign of toxicity showing that the water concentration for that chemical was unsafe. Therefore, the next lower concentration which did not cause a detectable molecular change was considered to be a "no-effect" (safe) level. These water concentrations for methylmercury, cadmium, and lead are shown as molecular indices

[4] Unipettes by Becton Dickson Co., Rutherford, N.J.
[5] Cyanmethemoglobin certified standard, Hycel, Inc., Houston, Tex.
[6] Monitrol-II control serum, Dade Reagents, Inc., Miami, Fla; and Versatol (normal) and Versatol-E (elevated) control sera, General Diagnostics Div., Warner–Chilcott Lab., Morris Plains, N.J.

TABLE 1

WEIGHT, LENGTH, HEMOGLOBIN, AND BLOOD PLASMA SODIUM VALUES OF ADULT BROOK TROUT EXPOSED TO METHYLMERCURIC CHLORIDE, CADMIUM CHLORIDE, AND LEAD NITRATE FOR 2 AND 8 WEEKS

Toxicant (μg of metal/liter)[a]	Weight (g)	Length (cm)	Hemoglobin (g/100 ml)	Sodium (mequiv/liter)
2-week exposure				
Methylmercuric chloride				
0.03 (control)[b]	64.98 ± 12.98 (20)[c]	18.36 ± 1.167 (20)	7.38 ± 0.86 (20)	143.89 ± 4.62 (19)
2.93	68.18 ± 14.95 (20)	18.59 ± 1.37 (20)	8.36 ± 1.99 (20)[d]	149.04 ± 6.77 (20)[d]
Cadmium chloride				
0.06 (control)	63.20 ± 10.52 (20)	18.22 ± 0.98 (20)	7.29 ± 1.10 (20)	144.31 ± 9.33 (19)
0.90	—	—	—	151.33 ± 7.53 (18)
6.35	61.24 ± 14.92 (20)	17.81 ± 1.33 (20)	7.50 ± 0.54 (20)	144.20 ± 8.45 (20)
Lead nitrate				
0.90 (control)	63.14 ± 12.29 (19)	18.31 ± 1.07 (19)	7.86 ± 0.97 (19)	110.15 ± 18.24 (19)[e]
58	—	—	6.73 ± 0.98 (20)[f]	
119	—	—	6.64 ± 0.99 (20)[f]	
235	—	—	6.81 ± 0.99 (21)[f]	
474	64.78 ± 15.62 (19)	18.28 ± 1.41 (20)	7.20 ± 0.72 (19)	134.78 ± 11.62 (19)
8-week exposure				
Methylmercuric chloride				
0.03 (control)[g]	100.62 ± 18.97 (19)	20.50 ± 1.29 (19)	6.62 ± 0.78 (19)	154.42 ± 11.50 (19)
2.93	100.71 ± 18.32 (20)	20.57 ± 1.16 (20)	6.68 ± 0.63 (20)	152.57 ± 9.23 (19)
Cadmium chloride				
0.06 (control)	95.84 ± 18.19 (19)	20.10 ± 1.31 (19)	6.63 ± 0.71 (19)	149.94 ± 9.40 (19)
6.35	98.68 ± 23.99 (10)	20.10 ± 1.56 (10)	6.55 ± 0.67 (10)	150.80 ± 8.48 (10)
Lead nitrate				
0.90 (control)	92.06 ± 13.30 (20)	20.02 ± 0.90 (20)	7.21 ± 0.88 (20)	142.50 ± 9.52 (20)
58	—	—	6.48 ± 0.88 (20)[d]	140.95 ± 7.25 (20)[d]
235	—	—	6.38 ± 0.64 (20)[f]	—
474	95.58 ± 18.58 (20)	20.18 ± 1.32 (20)	6.18 ± 0.66 (20)[f]	155.40 ± 8.67 (20)[f]

[a] Although six different water concentrations of each toxicant were used, only hematological values from the control and the highest concentration are given, except where statistical significance was found.

[b] Mean from three measurements.

[c] Mean ± SD; number of fish sampled in parentheses.

[d] Statistically significant at $p < 0.05$, relative to control.

[e] This value is low as compared to other control values, although we had no detectable instrument failure or technical error. Values for the intermediate toxicant concentrations (34–235 μg/liter) were about that at 474 μg/liter.

[f] Statistically significant at $p < 0.01$, relative to control.

[g] Mean from eight measurements.

TABLE 2

BLOOD PLASMA CHLORIDE, GLUCOSE, GOT, AND LDH VALUES OF ADULT BROOK TROUT EXPOSED TO METHYLMERCURIC CHLORIDE, CADMIUM CHLORIDE, AND LEAD NITRATE FOR 2 AND 8 WEEKS

Toxicant (μg of metal/liter)[a]	Chloride (mequiv/liter)	Glucose (mg/100 ml)	GOT (IU/ml)[b]	LDH (mIU/ml)[c]
2-week exposure				
Methylmercuric chloride				
0.01 (control)[d]	121.85 ± 9.12 (20)[e]	155.30 ± 29.01 (20)	114.66 ± 19.56 (20)	321.50 ± 134.97 (16)
2.93	127.75 ± 5.65 (20)[f]	169.10 ± 85.16 (20)	119.75 ± 19.56 (20)	298.20 ± 70.72 (10)
Cadmium chloride				
0.06 (control)	119.36 ± 10.93 (19)	104.77 ± 15.92 (18)	119.56 ± 20.87 (18)	351.00 ± 15.01 (8)
6.35	125.65 ± 10.63 (20)	102.30 ± 22.62 (20)	108.71 ± 33.46 (19)	302.00 ± 95.82 (12)
Lead nitrate				
0.90 (control)	118.20 ± 16.13 (18)	125.50 ± 13.37 (20)	167.69 ± 49.64 (15)	245.67 ± 90.36 (12)
34		—	144.45 ± 28.70 (20)[g]	—
58		—	127.52 ± 25.90 (20)[f]	—
119		—	141.80 ± 17.23 (20)[f]	—
235	126.10 ± 11.42 (20)[f]	—	143.10 ± 17.63 (20)[g]	—
474	129.60 ± 9.48 (20)[f]	137.25 ± 22.24 (20)	142.50 ± 17.40 (18)[g]	297.60 ± 68.32 (10)
8-week exposure				
Methylmercuric chloride				
0.01 (control)[h]	129.79 ± 7.25 (19)	121.73 ± 15.10 (19)	180.38 ± 80.94 (19)	278.73 ± 97.82 (15)
2.93	132.89 ± 6.57 (19)	135.95 ± 21.68 (20)	230.44 ± 119.64 (20)	260.92 ± 61.98 (13)
Cadmium chloride				
0.06 (control)	124.10 ± 7.13 (19)	151.26 ± 33.32 (19)	118.74 ± 16.42 (19)	266.52 ± 53.79 (19)
1.65	—	134.38 ± 22.29 (20)[g]	—	411.57 ± 139.56 (19)[g]
3.40	132.30 ± 9.46 (20)[f]	132.00 ± 12.61 (20)[g]	—	—
6.35	124.40 ± 6.77 (10)	154.60 ± 25.19 (10)	110.03 ± 18.37 (10)	306.00 ± 107.88 (10)
Lead nitrate				
0.90 (control)	129.89 ± 5.32 (19)	125.50 ± 13.37 (20)	116.35 ± 23.30 (20)	292.85 ± 100.33 (20)
58	—	—	97.91 ± 17.78 (17)[f]	—
235	—	—	93.51 ± 15.40 (20)[f]	—
474	129.95 ± 10.43 (20)	137.35 ± 22.14 (20)	106.87 ± 20.15 (20)	335.70 ± 117.25 (20)

[a] Although six different water concentrations of each toxicant were used, only hematological values from the control and the highest concentration are given, except where statistical significance was found.
[b] International units per milliter of blood plasma.
[c] Milli-international units per milliter of blood plasma.
[d] Mean from three measurements.
[e] Mean ± SD; number of fish sampled in parentheses.
[f] Statistically significant at $p < 0.01$, relative to control.
[g] Statistically significant at $p < 0.05$, relative to control.
[h] Mean from eight measurements.

in Table 3. Because we may not describe here the best biochemical parameters nor the most preferred time span for the evaluation of these toxicants, the molecular indices so listed in Table 3 are only provisionally assigned. The best biochemical indicators of distress from water pollutants will have to be developed from further studies on blood and tissue changes and particularly from studies concerned with the mechanism of

TABLE 3

MAXIMUM ACCEPTABLE SAFE CONCENTRATION INDICES FOR BROOK TROUT EXPOSED TO METHYLMERCURIC CHLORIDE, CADMIUM CHLORIDE, AND LEAD NITRATE[a]

Index	Water pollutant		
	Methylmercury	Cadmium	Lead
Molecular index (maximum concentration causing no significant biochemical change)			
Embryo[b]	0.08	3.30	530
Alevin-fry[b]	0.08	0.04	0.57
Adult[c]			
2-week exposure	0.94	6.35	0.50
8-week exposure	2.93	0.50	34
Bioassay index (maximum concentration causing no mortality, growth change, or physical deformity)[d]			
Alevin-fry	0.29	1.70	58
Adult, 38-week exposure	0.29	1.70	58
Residue index (maximum concentration showing no continuing increase of deposition in fish tissue)[d,e]			
Adult, 38-week exposure	0.01	0.50	0.90
Criteria index (maximum allowable water concentration in the U.S.)[f]			
For domestic water supplies	2.00	10.0	50
For fresh water aquatic life (approx., in soft water)	0.05	0.40	50

[a] Values are expressed as micrograms of metal per liter; statistically significant at $p < 0.05$.
[b] Christensen (1975); based upon the analysis of weight, ATP, protein, GOT, alkaline phosphatase, and acetylcholinesterase. The alevin-fry were exposed from the time of egg deposition.
[c] After 2- and 8-week exposures; based upon weight, length, hemoglobin, and blood plasma sodium, chloride, glucose, GOT, and LDH.
[d] McKim *et al.* (1976); Benoit *et al.* (1976); Holcombe *et al.* (1976).
[e] The tissues analyzed were spleen, kidney, liver, gill, brain, muscle, and erythrocytes.
[f] USEPA (1976).

action of classes of chemical pollutants. When the details of a developing toxicity are clearer than at present and we can understand cause–effect relationships better at the molecular, tissue and organ levels, we will be able to diagnose and prognose with improved assurance.

As seen in Table 3, a summary is presented from the biochemical results and a comparison is made between "no-effect" indices derived from other types of experimentation and calculations (viz., molecular, bioassay, residue, and water criteria).

The index values so listed for each chemical are considered to be a maximum "no-effect" concentration for *S. fontinalis* (an approximately "safe" value just preceding a threshold toxicity concentration).

Except for embryos exposed to lead (Table 3), the biochemical analyses provided indices of stress after 2–8 weeks, in contrast to bioassay values derived after 38 weeks of exposure. Changes in alevin-fry can be determined after even a shorter exposure time (and at relatively low toxic dosages), by way of both biochemical tests (Christensen, 1975) and bioassay studies on intact fish (McKim, 1977). Indeed, McKim has shown here that, for many water pollutants and several fish species, the embryo–fry life stage is the most susceptible stage and that bioassay data derived with these test animals are approximately equivalent to those obtained from year-long studies with all life stages from embryos through adults.

The induced biochemical changes in adults (Table 3) occurred roughly at concentrations causing biological changes as determined by chronic exposure tests. It is not yet clear where in time the reversible–irreversible threshold stage occurs; that is, the stage in exposure and metabolic effect whereby the animal can tolerate no higher toxic stress nor increase in the toxicant stressing agent. This should become elucidated if the defense systems of fish can be thoroughly studied and if detoxification, adaptation, and neutralization mechanisms are clearly revealed. Under exposure to toxicants, many molecular and biochemical changes in fish surely precede cellular and systemic changes, so that, if the appropriate biochemical factors are monitored, early warning signs of distress could be detected. This presumes that there is adequate knowledge of the mechanism of action so that casual relationships and molecular perturbations are at least approximately known and that adaptive and defense mechanisms are taken into account.

It was noted that there is an inconsistent dose–response relationship for some sets of results, which may be related to the variability of the members of the sample. In comparing biochemical values of control fish (brook trout) from many different experiments over the last several years, we have noted unexplainable differences in mean and variability values, except to suspect that fish develop certain metabolic responses from being held captive and that the responses may change somewhat depending on the exact circumstances of the captivity. For the application of anti-pollution toxicity studies it would be highly desirable to have baseline biochemical and hematological data from wild fish so that reliable extrapolations could be made from laboratory studies to the field situation.

The highest concentration of each of the toxicants studied here was ultimately found to be unsafe to the species as shown by a slow but steady mortality rate for fish exposed over a period of about 1 year.

These data provide further evidence that correlations can be made between molecular changes and gross biological changes in fish exposed to specific water pollutants, and that biochemical indices of toxicity can be derived after a relatively short exposure time which may be useful in predicting the approximate threshold toxicity concentration for chronic exposures.

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REFERENCES

AMES, B. N. (1977). The detection of environmental mutagens/carcinogens. *Fed. Proc.* **36**, 4.

BABSON, A. L., SHAPIRO, P. O., WILLIAMS, P. A., AND PHILLIPS, G. E. (1962). The use of diazonium salt for the determination of glutamic–oxalacetic transaminase in serum. *Clin. Chim. Acta* **7**, 199–205.

BENOIT, D. A., LEONARD, E. N., CHRISTENSEN, G. M., AND FIANDT, J. T. (1976). Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). *Trans. Amer. Fish. Soc.* **105**, 550–560.

BRIDGES, B. A. (1976). Short term screening tests for carcinogens. *Nature (London)* **261**, 195–200.

CHRISTENSEN, G. M. (1975). Biochemical effects of methylmercuric chloride, cadmium chloride, and lead nitrate on embryos and alevins of the brook trout, *Salvelinus fontinalis*. *Toxicol. Appl. Pharmacol.* **32**, 191–197.

CHRISTENSEN, G. M., MCKIM, J. M., BRUNGS, W. A., AND HUNT, E. P. (1972). Changes in the blood of the brown bullhead (*Ictalurus nebulosus* LeSuer) following short and long term exposure to copper (II). *Toxicol. Appl. Pharmacol.* **23**, 417–427.

FRANKEL, S., REITMAN, S., AND SONNENWIRTH, A. C., (eds.) (1970). In *Gradwohl's Clinical Laboratory Methods and Diagnosis*, 7th ed., Vol 1. C. V. Mosby, St. Louis, Mo.

HOLCOMBE, G. W., BENOIT, D. A., LEONARD, E. N., AND MCKIM, J. M. (1976). Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Canad.* **33**, 1731–1741.

KOEMAN, J. H., AND STRIK, J. J. T. (1975). *Sublethal Effects of Toxic Chemicals on Aquatic Animals*. Elsevier, New York.

MCKIM, J. M. (1977). Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J. Fish. Res. Bd. Canad.* **34**, 1148–1154.

MCKIM, J. M., CHRISTENSEN, G. M., AND HUNT, E. P. (1970). Changes in the blood of brook trout (*Salvelinus fontinalis*) after short-term and long-term exposure to copper. *J. Fish. Res. Bd. Canad.* **27**, 1883–1889.

MCKIM, J. M., OLSON, G. F., HOLCOMBE, G. W., AND HUNT, E. P. (1976). Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution, and elimination. *J. Fish. Res. Bd. Canad.* **33**, 2726–2739.

MUUL, I., HEGYELI, A. F., AND DACRE, J. C. (1976). Toxicological testing dilemma. *Science* **193**, 834.

NELSON, N. (Chairman) (1975). *Principles for Evaluating Chemicals in the Environment*, p. 5. National Academy of Science, Washington, D.C.

PALMES, E. D. (1976). Measurement and significance of subclinical effects of chemicals. *Clin. Toxicol.* **9**, 723–730.

Perkin–Elmer Corp. (1971). *Clinical Methods for Atomic Absorption Spectroscopy*. Sec. AA-Na-I. Perkin–Elmer Corp., Norwalk, Conn. 06852.

STEEL, R. G., AND TORRIE, J. H. (1960). *Principles and Procedures of Statistics with Special Reference to Biological Sciences*. McGraw–Hill, New York.

Technicon Corp. (1971). *Method for Glucose*, No. N-2b; *Method for LDH*, No. AA-21 (th-11-340 nm). Technicon Instrument Co., Tarrytown, N.Y. 10591.

THOMAS, W. A., GOLDSTEIN, G., AND WILCOX, W. H. (1973). *Biological Indicators of Environmental Quality*. Ann Arbor Science, Ann Arbor, Mich.

U.S. Environmental Protection Agency (1976). *Quality Criteria for Water*. EPA-440/9-76-023. USEPA, Washington, D.C. 20460.